



<i>Title:</i> AOS Protocol and Procedure: ZOO - Zooplankton Sampling in Lakes		<i>Date:</i> 10/22/2019
<i>NEON Doc. #:</i> NEON.DOC.001194	<i>Author:</i> S. Parker	<i>Revision:</i> J

AOS PROTOCOL AND PROCEDURE: ZOO - ZOOPLANKTON SAMPLING IN LAKES

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	05/28/2014	ECO-01127	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
C	11/14/2014	ECO-02467	Migration to new protocol template
D	05/15/2015	ECO-02666	Minor updates including changes to the number of integrated samples taken, concentration of ethanol, updates to sample shipping and labeling, and the addition of sampling dates to appendix.
E	01/21/2016	ECO-03448	Removed Secchi/depth profile details, updates to Schindler sampling depth.
F	02/08/2017	ECO-04359	Update NEON template; Update sample ID template; Add DNA metabarcode SOP
G	02/13/2018	ECO-05297	Tape jar lids prior to shipping, add barcode label information, update decontamination protocol reference, move datasheets to appendix
H	12/19/2018	ECO-05968	Update barcode info, clarify DNA sterilization, lab processing, and storage
J	10/22/2019	ECO-06243	Change lab processing time to 24 hours, individually bag DNA samples, new template



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1 OVERVIEW

1.1 Background

Zooplankton inhabit all layers of a water body and constitute a major link between primary production and higher trophic levels in aquatic ecosystems. Unlike algae or phytoplankton, zooplankton are microscopic animals that do not produce their own food (Figure 1). These small invertebrates float freely in the water column of lakes and oceans. They are important as both prey and consumers in the aquatic food web. Zooplankton are often filter feeders (sometimes predators) that feed primarily on algae while also being the main food source for planktivorous fish. The zooplankton assemblage responds to environmental stressors such as nutrients, and such effects can be detected through changes in species composition, abundance, and body size distribution (USEPA, 2012).

Many zooplankton are capable of strong swimming movements and may migrate vertically from tens to hundreds of meters; others have limited mobility and depend more on water turbulence to stay afloat. Zooplankton can be classified according both to developmental stages: meroplankton and holoplankton; and to size (Sanders and Porter, 1990). Meroplankton spend only part of their life cycles as plankton, while holoplankton spend essentially their whole existence in the water column. Freshwater zooplankton are dominated by six major groups of animals: protozoa, rotifers, two subclasses of the Crustacea, the cladocerans and copepods, and *Mysis* and *Chaoborus* (Figure 1 a-d). The planktonic protozoa in particular have limited locomotion and are dominated by the meroplanktonic pelagic zooplankton (5 – 300 μm), characterized by a summer planktonic lifecycle, spending the rest of their life cycle in the sediments. Several non-planktonic rotifers (150 μm – 1 mm) are sessile (lower mobility) and mostly associated with the littoral zone (Figure 2). Most rotifers are non-predatory, and omnivorously feed on bacteria, small algae, and detrital particulate organic matter. The majority of Cladocera are small (0.2 to 3.0 mm) and have a distinct head and bivalve carapace covering the body. Locomotion is accomplished mainly by means of the large secondary antennae. Planktonic copepods (2-4 mm) consist of two major groups, the calanoids (*Diatomus*) and the cyclopoids, distinguished by their body structure and length of antennae.

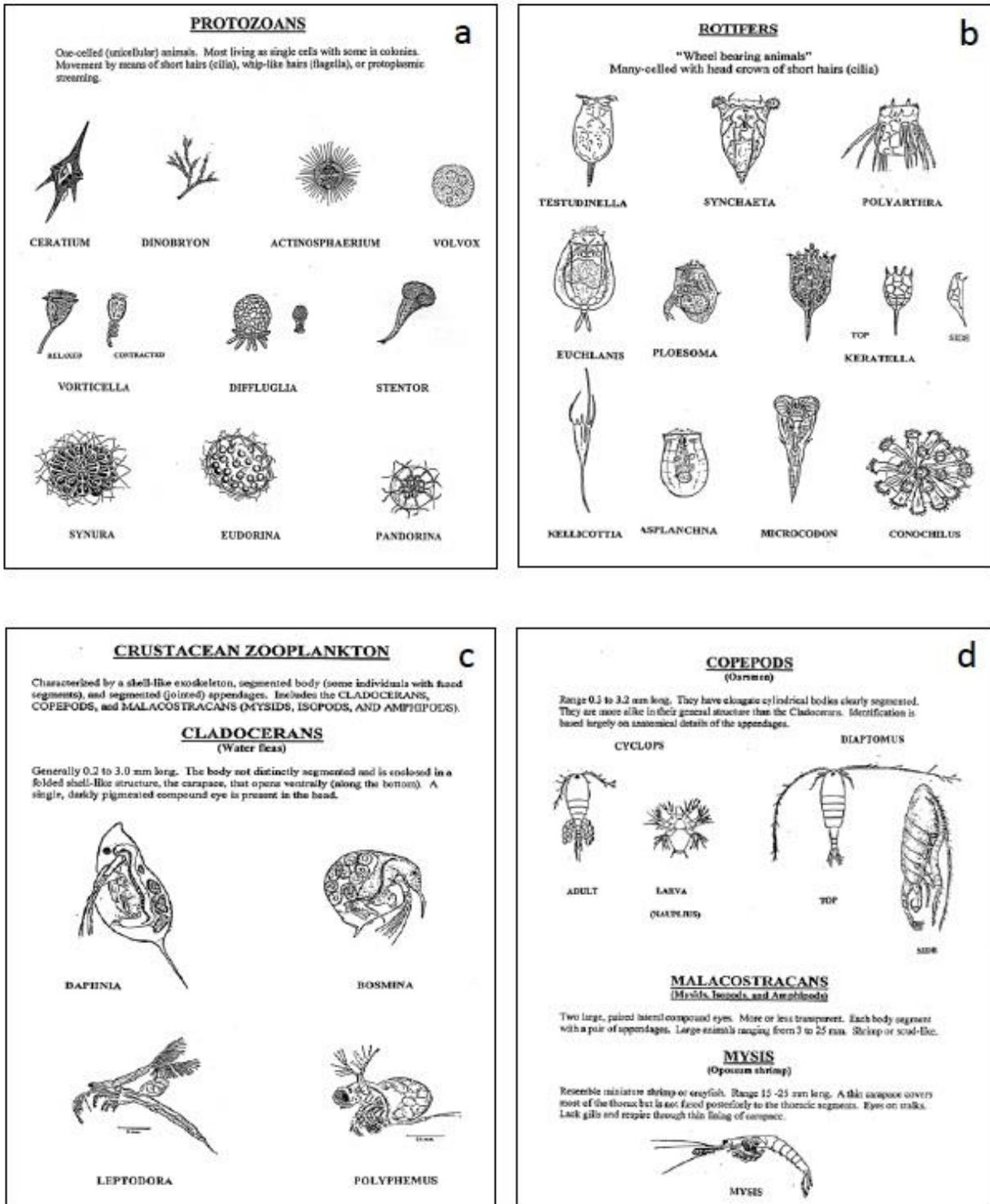


Figure 1. Classes of zooplankton (from Tharp).

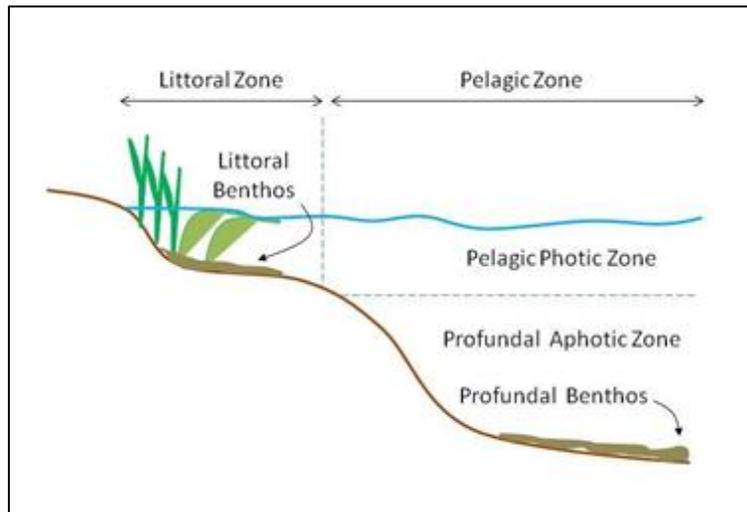


Figure 2. Zones of a lake.

Seasonality plays an important role in zooplankton species presence and abundance, with cyclopoid and calanoid copepods dominating the community in the winter months, with large cladocera peaking in mid-summer and small cladocera in later summer early autumn (Gerten and Adrian, 2002). A change in lake trophic conditions from oligotrophic to eutrophic has been shown to result in size-selective predation that potentially contributes to the decline of larger zooplankton, with ciliated protozoans and rotifers becoming more important (Wetzel, 2001).

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

This protocol is based on modified version of USEPA (2012a, b), Baker et al. (1997), and USEPA (2009).



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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHSS Policy, Program and Management Plan
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.002302	Datasheets for AOS Protocol and Procedure: Zooplankton Sampling in Lakes
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[09]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of sensors, field equipment and field vehicles
RD[10]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[12]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Depth and Depth Profile Sampling
RD[13]	NEON.DOC.014050	TOS Protocol and Procedure: Ground Beetle Sampling
RD[14]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[15]	NEON.DOC.003600- NEON.DOC.003618	Aquatic Site Sample Design – NEON Domain ##
RD[16]	NEON.DOC.005224	Shipping Ecological Samples, Sensors and Equipment



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2.3 Acronyms

Acronym	Definition
DI	Deionized
EMAP	Environmental Monitoring and Assessment Program (USEPA)
mL	milliliter
NLA	National Lakes Assessment (USEPA)
PFD	Personal Flotation Device
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

2.4 Definitions

Epilimnion: Top layer of water of a stratified lake, denoted by highest temperatures and least dense water. Typically occurs in the summer.

Euphotic zone (or “Photic zone”): The upper layer of lake water where sunlight penetrates and photosynthesis can occur. Specifically, the depth to which 1% of surface light penetrates.

Eutrophic: An ecosystem with high nutrient concentration. In lakes, this often equates to algal proliferation or algal blooms.

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is cooler than the surface water and has less circulation.

Metalimnion: The layer of water in a stratified lake that sits between the hypolimnion and the epilimnion. Often equated with the thermocline.

Oligotrophic: An ecosystem with low nutrient content. In lakes, this often equates to very clear water and little algal production.

Pelagic: The part of the lake that is not near shore or close to the bottom.

Stratified: Layers within the system (e.g., warm and cold water layers indicate thermal stratification in a lake).

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1 °C per meter. The denser and cooler layer below the thermocline is the hypolimnion, the warmer upper layer is termed the epilimnion.



3 METHOD

The goals of the Zooplankton Sampling in Lakes Protocol are to quantify biodiversity, number of species present, and biomass (or biovolume) at each lake site. These variables will be used to build a database over time, in order to track changes in zooplankton community structure and function, and introduction of invasive species.

Samples shall be collected as an integrated water column sample at the central location of the lake. Two additional samples are taken at the inlet and outlet of the lake or a location downwind of the predominant wind direction. Samples are collected 3 times per year in order to capture differences in community composition, abundance, diversity, and biomass.

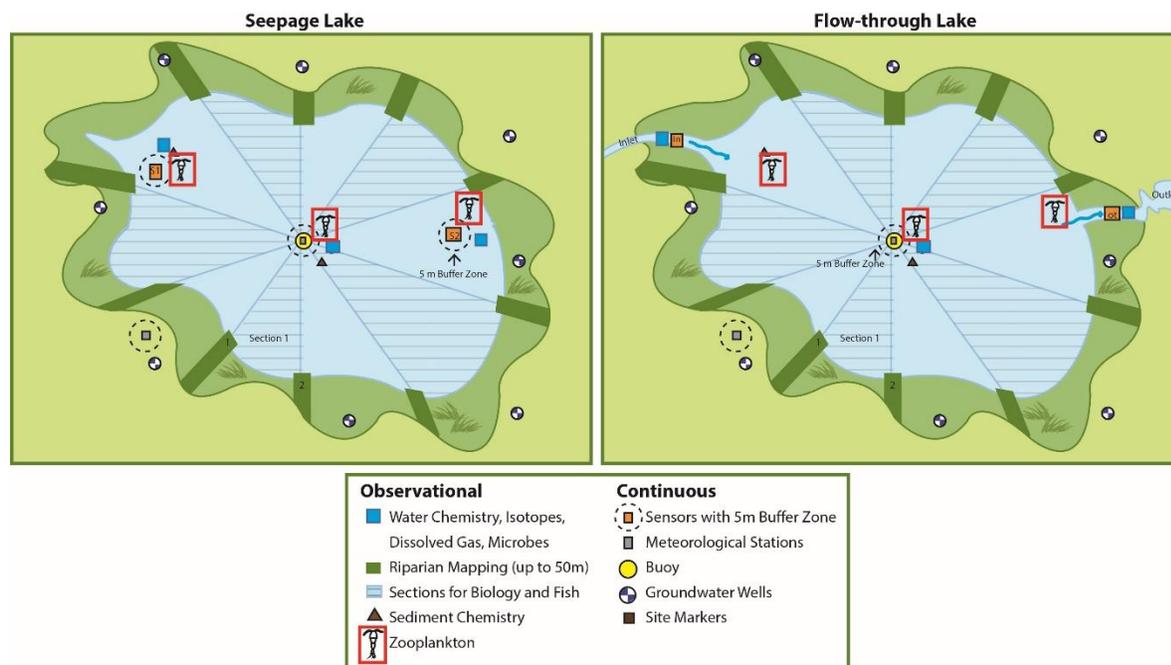


Figure 3. A generic lake site layout with zooplankton sampling locations. Seepage lakes have no true inlet or outlet stream. In flow-through streams, inlet and outlet infrastructure are located in the inlet or outlet stream channel.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field ecologists **must** follow the protocol and associated SOPs. Use NEON’s problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that field ecologists document the problem and enter it in NEON’s problem tracking system.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Lake zooplankton sampling occurs three times per year at each lake site, roughly spring, summer, and autumn (Appendix C). Sampling must be initially scheduled within the first 21 days of the 1 month window specified in Appendix C with a minimum of two weeks between sampling dates.

Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall later in the bio bout or outside of the pre-determined window. See the appropriate Aquatic Site Sampling Design for your domain for additional details and scheduling preferences. Use NEON’s problem reporting system to seek guidance and report sampling efforts that take place outside of the defined sampling window.

Zooplankton sampling takes place on the same day as Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams (RD[12]).

Table 1. Sampling frequency for zooplankton procedures on a per SOP per site type basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B	lake	buoy, inlet, outlet	1 day	3	Minimum of 2 weeks between bouts	annual	SOP B and C must be done on the same day
SOP C	lake	buoy, inlet, outlet	1 day	3	Minimum of 2 weeks between bouts	annual	Bout 2 samples sent for analysis, Bout 1 and 3 samples sent to archive

Scheduling Considerations

1. All samples for a bout must be collected during the same day (includes SOP B and SOP C).
2. **Field Work and Laboratory Processing:** After zooplankton samples are collected, the following points are critical with respect to timing:



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- a. Preserve samples in the field within 30 minutes of collection.
- b. Keep samples cool (4 °C) during transportation and storage.
- c. Replace ETOH in DNA samples within 24 hours of collection.
- d. Store all samples at 4 °C until shipping.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including ice on/ice off, the accumulation of degree days, weather, and riparian phenology (Appendix C).

4.3 Timing for Laboratory Processing and Analysis

Zooplankton DNA samples must have their preservative changed within 24 hours of field collection. Though samples should ideally be shipped to external processing facilities within 30 days of collection, preserved and refrigerated samples may be held longer at the domain support facility if necessary.

4.4 Sample Timing Contingencies

All samples from this protocol in one sampling bout must be collected within one day (i.e., all samples per lake/non-wadeable stream as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases data variability among samples. Sampling may be rescheduled due to weather or environmental conditions within the 10 days of the scheduled date provided that date is within the bout window (+ 3 days) provided in Appendix C. An incident ticket must be issued if sampling is rescheduled >10 days past the original scheduled date, or >3 days past the end of the bout window in Appendix C. A minimum of 2 weeks between sample periods shall be observed.



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Table 2. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and the lake becomes unsafe (e.g., approaching thunderstorm) or becomes too windy (>35 km hr ⁻¹⁺ _[small]) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes. If conditions improve, resume sampling, if not, discard samples, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data may be flagged.
6 Months	Preserved zooplankton samples may be held for up to 6 months at 4 °C in the domain support facility if circumstances do not allow shipping to the external laboratory.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more sampling locations in a given bout. For example:

- Environment – e.g., ice cover, inclement weather, or
- Management activities – e.g., controlled burns, herbicide application

Instances such as those listed above must be documented for scheduling, tracking long-term site suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[14]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates:** Bout-specific sampling dates suggested by the protocol and site-specific sample designs (Appendix C and RD[15]).
- **Scheduled Sampling Dates:** Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling:** Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded (e.g., each missed zooplankton sample gets its own record).



- **Sampling Impractical:** The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled:** Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (Figure 4).

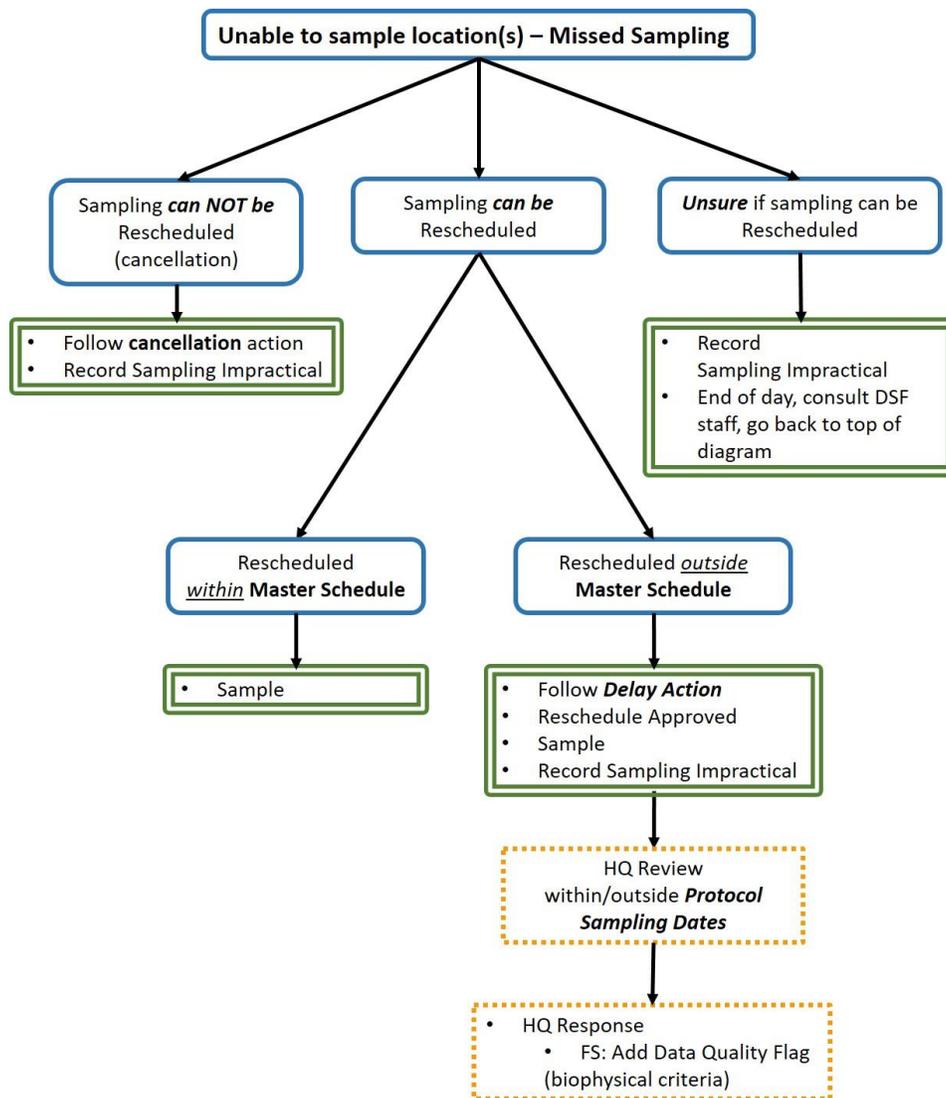


Figure 4. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Blue rounded boxes represent contingencies, green double line boxes describe the required actions, Orange dotted boxes indicate HQ actions. Required delay and cancellation actions are outlined for each protocol in the ‘Scheduled Field Activities – Delays and Cancellations’ spreadsheet available on the SSL.



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Missed Sampling events may also require a Data Quality flag and/or creation of a Site Management record.

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling must be communicated to Science by a Service Now Incident.
 - a. For **Missed Sampling** that is **Rescheduled**, there are some cases that require approval by Science and Operations (Figure 4).
 - b. The lead Field Ecologist should consult the [Delayed or Cancelled Activities table](#) to best determine when reporting is required.
2. Create a field record for each **Missed Sampling** event in the field. That is, if data are recorded in the field at the site level, a record must be made.
 - a. Example: Lake is ice covered and sampling cannot be attempted.
3. For each **Missed Sampling** record, the **Sampling Impractical** field must be populated in the mobile collection device (Table 3).
 - a. Three zooplankton taxonomy samples should be collected during each sampling event, else sampling should be re-attempted.
4. For **Rescheduled** sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Data Quality Flag must also be recorded (Figure 4).
 - a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions
 - b. windAffected – An indicator of whether winds were >35 km hr⁻¹ at the time of sampling

Table 3. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
Location dry	Location dry
Location frozen	Location frozen
Location snow covered	Location snow covered
Other	Sampling location inaccessible due to other ecological reason described in the remarks



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4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, use NEON’s problem reporting system to notify Science. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Field sampling requires two field ecologists for four hours per site plus travel to and from the site. There is 1 hour of domain lab processing for 1 person when DNA samples are collected. There is no domain lab processing associated with taxonomy samples in this protocol.

Table 4. Estimated staff and labor hours required for implementation of Zooplankton Collection

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1 and A.2: Preparing for data collection and sampling	1 h	1	1 h
SOP B: Zooplankton taxonomy collection	3 h	2	6 h
SOP C.1: Sterilize DNA equipment	1 h	1	1 h
SOP C2: Zooplankton DNA collection	1 h	2	2 h (combined with SOP B)
SOP D: Laboratory preservative swap (DNA)	1 h	1	1 h
SOP E: Data Entry and Verification	1 h	1	1 h
SOP F: Shipping	1 h	1	1 h



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5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Ecologist have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Safety Data Sheets (SDS) shall be readily available and reviewed for all chemicals (ethanol) used during this task. Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) for Laboratory Safety and NEON EHSS Policy, Program and Management Plan (AD[01]).

See Section 9 in the NEON Operations Field Safety and Security Plan (AD [02]) for aquatic-specific field safety requirements. In addition, the following safety requirement must be followed:

1. In lakes, site-specific hazards may be encountered may necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
2. All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
3. All personnel shall have access to a form of communication with other team members such as a two-way radio.
4. Be aware of any site-specific hazards and to the waters of that particular location (i.e., current status, tidal charts, etc.).



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6 PERSONNEL

6.1 Training Requirements

Field staff must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

All personnel required to operate a boat shall be trained through an approved program. All others shall be aware of boating safety procedures.

Personnel will be trained in field protocols associated with this document, and trained in safe working practices for aquatic field work. Personnel must also be trained in safe handling of ethanol (AD[03]).

6.2 Specialized Skills

Where applicable, personnel will be licensed to operate a boat and able to safely handle a motor and drive a boat safely.



7 STANDARD OPERATING PROCEDURES

SOP Overview

- SOP A: Preparing for Sampling
- SOP B: Field Sampling
- SOP C: Zooplankton DNA Metabarcoding Field Collection
- SOP D: Post-Field Sampling Tasks
- SOP E: Laboratory Sampling and Analysis
- SOP F: Data Entry and Verification
- SOP G: Sample Shipment

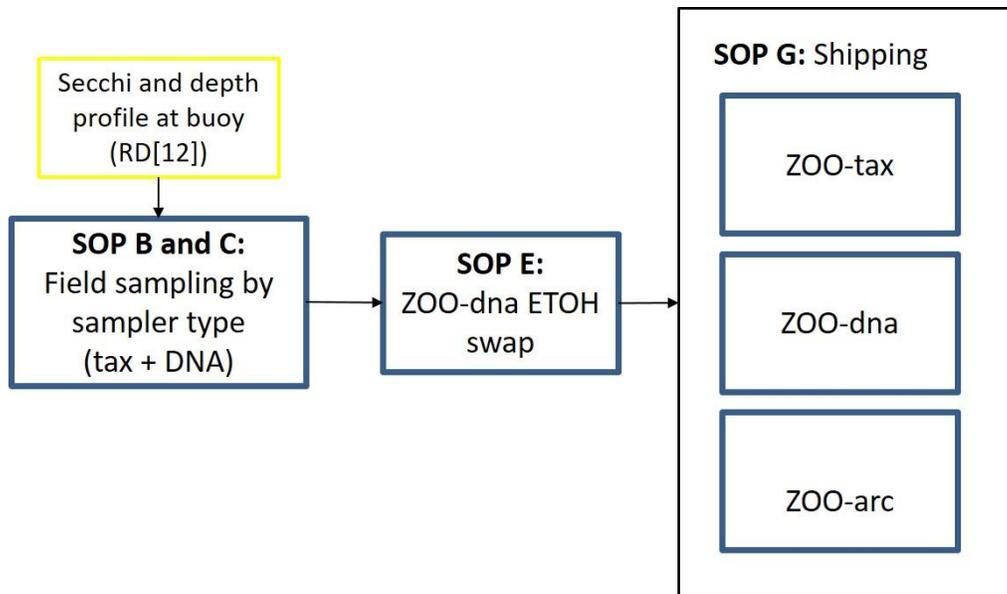


Figure 5. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.



3 ZOO-tax

human-readable label +
Type I barcode on every
bottle

3 ZOO-dna

Figure 6. Zooplankton sample suite, including 3 ZOO-tax and 3 ZOO-dna bottles with human readable and barcode labels.

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged and synced at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Labels and Identifiers

All barcodes need to be applied to dry containers for 30 mins before use. Zooplankton collection uses Type I barcodes (prefix A, plus 11 numbers). Type I barcodes are for all field samples and any non-cryo applications; they have a tolerance from 4C to 105C and still scan.

1. All zooplankton samples will have a weather-resistant, adhesive, human readable label (Figure 7).
2. Adhesive barcode labels should be applied to dry, room temperature zooplankton bottles in advance of their use in the field, at least 30 minutes prior (Figure 8).
 - a. Barcode labels should be aligned lengthwise along the bottle as the scanner will not work on a curved surface (i.e. horizontally wrapped around the bottle; Figure 6).
 - b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially



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associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

<p>NEON <input type="checkbox"/> DNA <input checked="" type="checkbox"/> ETOH preserved</p> <p>Sample ID: <u>BARC.20161003.towNet.1</u></p> <p>Sample type: <u>tow net</u> Schindler-Patalas</p> <p>Location: <u>c0</u></p> <p>Number of tows/traps: <u>2</u></p> <p>Collected by: <u>sparker</u></p>	<p>NEON <input type="checkbox"/> DNA <input checked="" type="checkbox"/> ETOH preserved</p> <p>Sample ID: <u>BARC.20161003.schindler.2</u></p> <p>Sample type: tow net <u>Schindler-Patalas</u></p> <p>Location: <u>in</u></p> <p>Number of tows/traps: <u>2</u></p> <p>Collected by: <u>sparker</u></p>
<p>NEON <input checked="" type="checkbox"/> DNA <input checked="" type="checkbox"/> ETOH preserved</p> <p>Sample ID: <u>BARC.20161003.towNet.1.DNA</u></p> <p>Sample type: <u>tow net</u> Schindler-Patalas</p> <p>Location: <u>c0</u></p> <p>Number of tows/traps: <u>2</u></p> <p>Collected by: <u>sparker</u></p>	<p>NEON <input checked="" type="checkbox"/> DNA <input checked="" type="checkbox"/> ETOH preserved</p> <p>Sample ID: <u>BARC.20161003.schindler.2.DNA</u></p> <p>Sample type: tow net <u>Schindler-Patalas</u></p> <p>Location: <u>in</u></p> <p>Number of tows/traps: <u>2</u></p> <p>Collected by: <u>sparker</u></p>

Figure 7. Example of human-readable adhesive field labels for zooplankton sampling.



Figure 8. Example of adhesive barcode labels, Type I. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.



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Table 5. Sample types and barcodes used.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
ZOO-tax	Preserved zooplankton sample	PRPO.20161027.townet.1 OR PRPO.20161027.schindler.1	(AOS) Zooplankton Field Data [PROD]	500 mL HDPE bottle	Type I	Yes	1 per bottle, 3 per bout
ZOO-dna	Preserved zooplankton DNA sample	PRPO.20161027.townet.1.dna OR PRPO.20161027.schindler.1.dna	(AOS) Zooplankton Field Data [PROD]	500 mL HDPE bottle	Type I	Yes	1 per bottle, 3 per bout

A.3 Zooplankton Field Sampling

1. Collect and prepare all equipment, including sample bottles and labels.
 - a. Load GPS sampling coordinates in GPS (accuracy ± 4 m).
2. Pre-print human-readable adhesive labels (Figure 7) for sample bottles and data sheets on all-weather paper (label template are available in the Sampling Support Library or RD[05]).
 - a. Labels must be pre-printed or filled out using pencil or ethanol-safe pen in the field.
 - b. Use the following naming convention for sample IDs.
 - c. Sample IDs written on the physical sample label must match the sample ID generated by the mobile app: *SITE.YYYYMMDD.samplerType.sampleNumber[.DNA]*
 - i. SITE = 4 letter site code
 - ii. YYYYMMDD = Collection date
 - iii. samplerType = Type of sampling equipment used
 - iv. sampleNumber = Numbers 1-3, corresponds to one of three samples. Does not need to be directly linked to a specific location.
 - v. [DNA] = Append to sample ID if this is a DNA sample
3. Add Type I adhesive barcode labels to sample containers. Scan with the mobile app (Figure 11). Add labels to zooplankton bottles prior to going in the field and getting the bottle wet.
 - a. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.
4. Check for holes in nets, assure that nets are clean and free of debris and organic matter and have been decontaminated (RD[09]) since last use.
5. Have ice or ice packs frozen and ready for cooler.
6. Fill 1 L HDPE bottles (or 4 L HDPE jug) with 95% ethanol. Cap tightly. Clearly label bottle (suggest using Globally Harmonized System (GHS) labels) and transport to the field following EHS guidelines.
7. Check that all equipment is in good condition and all batteries are charged.
8. Enter general aquatic field metadata (RD[06]) and Secchi and depth profile (RD[07]) in their respective mobile applications once per day upon every field visit. If other protocols are done in the same day, one record for field metadata and one record for Secchi and depth profile are sufficient.



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SOP B Field Sampling

Data for field sampling are entered in the (AOS) Zooplankton Field [PROD] mobile application. Instructions for the use of this application can be found in the Sampling Support Library in the document “Manual for Fulcrum Application: (AOS) Zooplankton [PROD]”.

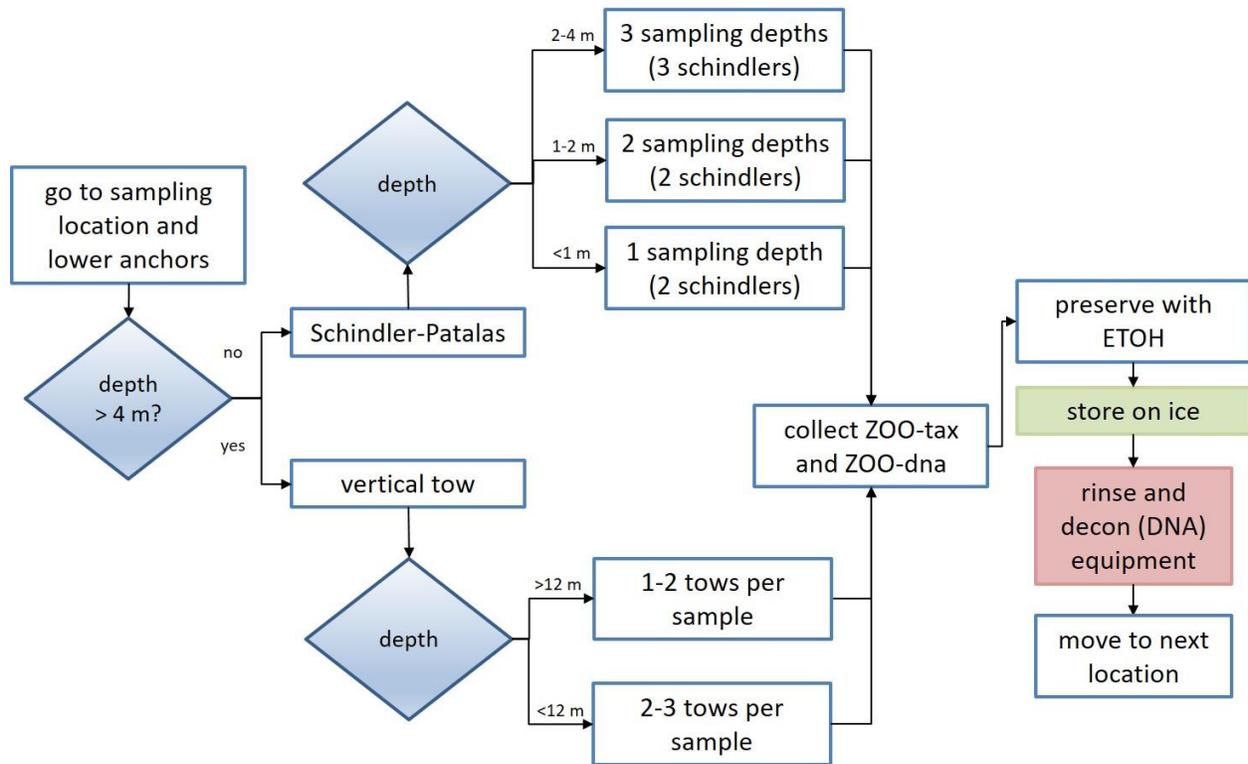


Figure 9. Workflow for field sampling SOPs for zooplankton collection.

B.1 Spatially and Temporally Linked Protocols

Field Metadata and Gauge Height

- Data are entered into the (AOS) Field Metadata and Gauge Height [PROD] app at the start and end of every aquatic field day.

Secchi Depth and Depth Profile Sampling

- Secchi and depth profile measurements must be recorded with every protocol that samples the water column, such as zooplankton (RD[12]).



B.2 Locating Sampling Locations

1. In the deepest point in the lake (near buoy), determined by site map and pre-loaded GPS coordinates.
 - a. Location ID = "c0" (lake not stratified) or "c1" (lake stratified)
2. Near the major inlet in the littoral zone.
 - a. Location ID = "in"
3. Near the outlet in the littoral zone.
 - a. Location ID = "ot"

B.3 Collecting Samples

Zooplankton taxonomy (ZOO-tax) samples are collected during each sampling bout, three times per year. Collecting three samples per bout is the minimum required to maintain data quality.

1. Navigate to the sampling location. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Gently lower anchors at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place.
 - a. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
 - b. Using a bow anchor rope 2 times the water depth will minimize disturbance of the sediment at the sampling location.
 - c. The boat must be anchored at the bow and stern so that the boat doesn't rotate, in order to collect representative water column samples.
3. Always sample near the bow of the boat to minimize the effects of the motor on the water column. When anchored, the boat tends to orient itself with the bow into the wind or current.
4. Determine the total water depth from depth finder readings or a weighted rope.
 - a. If depth is ≤ 4 m, or if phytoplankton clog the tow net, use a Schindler-Patalas sampler and proceed to Step 5.
 - b. If depth is >4 m, use the tow net and proceed to Step 7.
5. **Schindler-Patalas sampler** (Figure 10): Integrate 2 to 3 Schindler traps to equal 1 sample. Measure to the bottom of the Schindler trap box (Figure 12). Record sample type, sample depths, and number of traps collected in mobile application.
 - a. If depth is 2-4 m, integrate the following (3 traps):
 - i. 0.5 m below surface (measure to bottom of Schindler trap)
 - ii. Middle of water column
 - iii. 0.5 m above lake bottom
 - b. If depth is 1-2 m, integrate the following (2 traps):
 - i. 0.5 m below surface
 - ii. 0.5 m above lake bottom





- c. If depth is <1 m, integrate the following (2 traps):
 - i. Sample in locations that are deeper than 0.8 m to prevent disturbing the sediment contaminating the sample with benthic material.
 - ii. 2 Schindler traps 0.5 m below surface



Figure 10. Example of a Schindler-Patalas sampler with Dolphin bucket.

6. When using the Schindler-Patalas trap:
 - a. Ensure the dolphin bucket is attached (Figure 10)
 - b. Lower sampler to appropriate depth at a consistent speed so trap doors do not close prematurely.
 - i. When the depth is reached and the sampler stops descending, the trap doors will close automatically.
 - c. Bring the sampler to the surface. The water will drain through the net.
 - i. When filled with water, this sampler is heavy. Work gloves may be worn to protect hands when pulling in the rope.
 - ii. Lift the sampler slowly to allow water to drain through the net and dolphin bucket.
 - a) Once above the water's surface, opening the lid of the Schindler breaks the suction and allows water to flow into the dolphin bucket faster. Do not allow additional source water into the sampler at this point.
 - d. Inspect the sample for organic and inorganic debris. If sediments were captured, discard sample and start again. If organic debris (e.g., leaves, plants) is noted accounting for >25% of sample, discard sample and start again. Smaller amounts of organic debris can be removed by hand.
 - i. Discard sample away from where you intend to collect the next sample.
 - e. Rinse the sampler and net until the entire sample is in the dolphin bucket.
 - i. Lake water can be used on the outside of the net
 - ii. DI, tap water, or 63 μm mesh-filtered source water (note: this is not the same mesh size used for macroinvertebrate sampling) must be used if rinsing the inside of the trap or net to ensure that no additional zooplankton are added to the sample.





- f. Carefully remove the dolphin bucket and transfer sample to a 500 mL sample bottle. Rinse dolphin bucket into collection bottle. Samples volume may be significantly less than 500 mL.
- g. Repeat steps above to integrate Schindler-Patalas samplers.
- h. Record data in the mobile app.
 - i. Scan the barcode label with the tablet (Figure 11).
 - ii. Record the total number and sampling depths of the Schindler-Patalas samples added to the composite in the mobile app.
 - iii. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

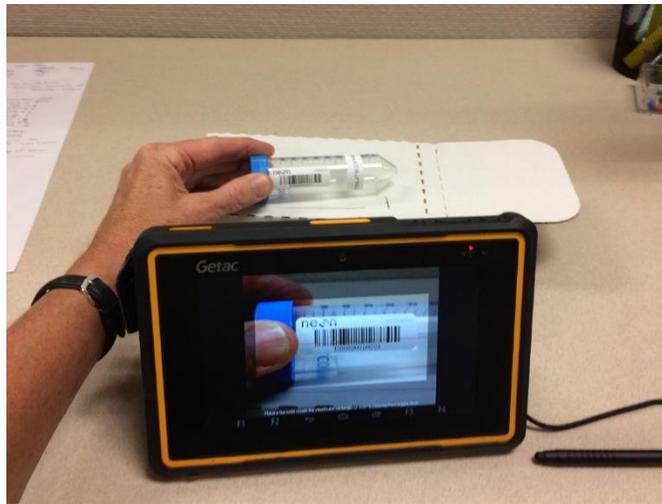


Figure 11. Barcode label scanning.

- i. Preserve according to Sample Preservation (Section B.4) and place sample bottle in cooler.
- j. Proceed to next sampling location, rinse trap, net, and dolphin bucket well with lake water, and repeat steps above.

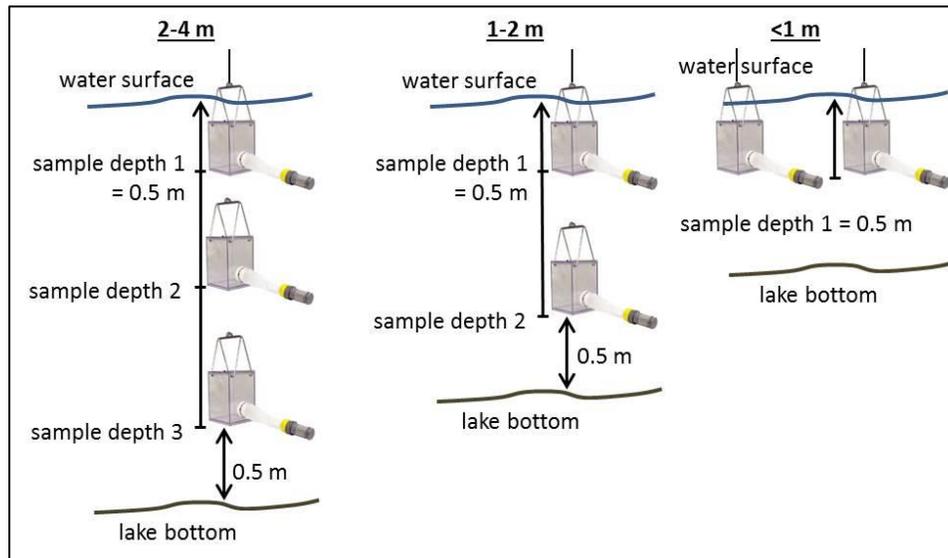


Figure 12. Schindler-Patalas sampling depths.

7. **Tow net sampler** (Figure 13): integrate at least 2 tow nets to equal 1 sample. Measure depth to the top of the tow net mouth. Note sample type, sample depths, and number of tows collected in the mobile app.
 - a. If the sampling location is >12 m deep, assess the amount of zooplankton collected after 1 tow. If zooplankton fill at least half of the sample bottle, you do not need to collect a second tow.
 - b. Ensure that the tubing end is firmly attached and clamp is closed over tubing prior to sampling. The tubing end comes loose easily.
 - c. Lower the net to where the bottom tubing is 0.5 m above the bottom of the lake. If conditions are windy, add a small weight at the bottom of the net to help lower the tow net.
 - d. Pull the nylon rope vertically at a rate of 0.5 m/s until top of net is out of the water.
 - e. Inspect the sample for organic and inorganic debris. If sediments were captured, discard sample and start again. If organic debris (e.g., leaves, plants) is noted accounting for >25% of sample, discard sample and start again. Smaller amounts of organic debris can be removed by hand.
 - i. Discard sample away from where you intend to collect the next sample.
 - f. If algae clog the net, rinse the sides of the net down with the 500 mL wash bottle. If water cannot drain through the net, consider using the Schindler-Patalas sampler at this location.
 - i. Lake water can be used on the outside of the net
 - ii. DI, tap water, or 63 μm filtered lake water must be used if rinsing the inside of the trap or net to ensure that no additional zooplankton are added to the sample.
 - g. After the inspection, slowly lower the net into the lake to within inches of the net opening above the water surface and abruptly pull upwards out of the water to collect the contents of the net at the bottom. *Take care not to submerge the top of the net opening below the water surface and introduce more sample water.* Repeat several times until the net is fully rinsed.





Figure 13. Zooplankton tow net with bottom tubing and clamp.

- h. Complete the rinsing of the net contents by spraying water against the **outside** of the net with a wash bottle filled with lake water. If rinsing the inside of the net, use DI, tap water, or 63 μm filtered lake water. Be sure to inspect and rinse the net seams carefully for trapped zooplankton.
- i. Holding the net in a vertical position, carefully open the spigot on the hose and pour into the 500 mL sample bottle.
 - i. If large organic debris is present, remove the large debris with forceps.
- j. Rinse the concentrated zooplankton into the collection bottle using a squirt bottle filled with DI.
- k. Enter the tow depth (distance in meters of water column through which the collecting unit was retrieved) in the mobile app (Figure 14) so sample volume can be calculated.
- l. Collect two or more tows following the steps above to increase the number of organisms in the sample.
 - i. Leave space in bottle for preservative (bottle should be no more than $\sim 1/2$ full before preservative is added).
- m. Record data in the mobile app.
 - i. Scan the barcode label with the tablet.
 - ii. Record the total number and sampling depths of the individual tow nets added to the composite in the mobile app.
 - iii. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.
- n. Preserve according to Sample Preservation (Section B.4) and place sample bottle in cooler.



- o. Proceed to the next sample location, and rinse the net, collection cup and retrieval rope thoroughly (2 to 3 times) with water from the site on the downwind side of the sampling location and repeat above steps.

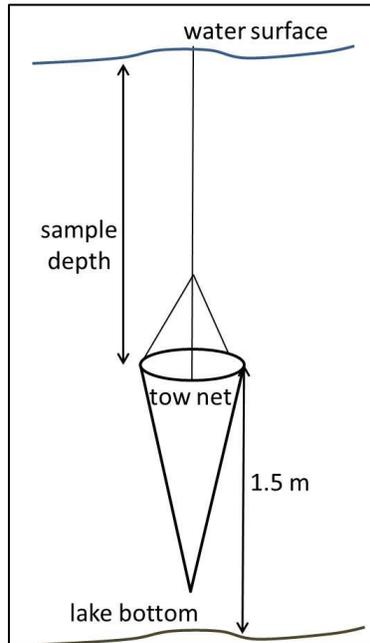


Figure 14. Tow net configuration.

B.4 Sample Preservation



All processing must be completed in the field. Should conditions not allow for this step to be undertaken in the field within 5 minutes of sampling, all preservatives shall be added within 30 minutes of collecting the sample in the field. If samples are not preserved within 30 minutes, discard and resample.

1. Add enough 95% ETOH solution to the 500 mL sample bottle within 5 minutes of collection to reach a final concentration of ~30-40% ETOH.
 - a. The sample can be <500 mL.
 - b. Consider using a larger sample bottle (1 L) if there is not enough room to add ethanol to reach a final preservative concentration of 30-40%.
 - c. Use $C_1V_1=C_2V_2$ to calculate preservative volume, where:
 - i. C_1 =concentration of ETOH before adding to sample (95%)
 - ii. V_1 =**volume** of ETOH before adding to sample
 - iii. C_2 =**concentration** of ETOH in final sample (30-40%)
 - iv. V_2 = **volume** of final sample



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- d. As a rule of thumb, add enough 95% ETOH to slightly less than double the sample volume (i.e., <50% ETOH in final sample).
2. Carefully re-cap bottle, ensuring that no sample escapes, and gently invert bottle to mix.
3. Store in a cooler (~4 °C) to return to Domain Support Facility.

SOP C Zooplankton DNA Metabarcoding Field Collection

Zooplankton DNA (ZOO-dna) samples are collected during each sampling bout, three times per year. Samples collected specifically for DNA metabarcoding will be analogs to three of the samples already being collected. This sampling approach uses the community metabarcoding approach, where replicate samples from the site are preserved in high-concentration ethanol for sequencing analysis. These samples are collected using field methods that are as “sterile” as possible, with the understanding that it is difficult to maintain sterility in the field. Equipment will be sterilized in the domain lab prior to sampling, and rinsed with source water between samples at a site on a given day.

C.1 Sterilize equipment

All equipment must be cleaned and sterilized prior to sample collection at the site to prevent contamination of DNA from the person collecting the sample, DNA from another site or sampling date, or DNA from the environment outside of the site (Laramie et al. 2015).

1. Cleaning equipment:
 - a. Wearing nitrile gloves, clean all nets and other equipment that comes in contact with samples during zooplankton sampling using a 10% bleach solution. Follow with a DI or tapwater rinse to remove the residual bleach (Jane et al. 2014, Laramie et al. 2015). Note that this is a higher concentration than is usually used in the Aquatic Decontamination Protocol (RD[09]).
 - b. Equipment to decontaminate with stronger solution:
 - i. Tow net
 - ii. Schindler-Patalas and dolphin bucket
 - iii. Collection bottles, if they do not come clean and capped from the lab
 - iv. Forceps, sieves, or any additional equipment that comes in contact with the sample
 - v. Waders and boots if you plan to wade near the sampling location. If you remain in the boat, this is not necessary.
2. Wearing clean nitrile gloves, place equipment and consumables in a clean bag so as not to contaminate it on the way to the field site.

C.2 Field collection

1. Fill out and place an adhesive label on the collection bottle. Check “DNA” on label.



2. Collect DNA samples after collecting taxonomy for consistency. Prioritize taxonomy sampling if contingent decisions are necessary.
 - a. Collect an additional sample at each sample location (inlet, outlet, and buoy) using the same sampler already chosen to use for taxonomy samples at each of those locations (SOP B). Wear clean nitrile gloves while collecting DNA samples.
3. Prior to sampling, prime the sampler by rinsing well with local source water. Rinse from the outside of the net so zooplankton and other organisms are not trapped inside the net during rinsing. You may use surface water (this adds minimal DNA to the sample when compared to the actual organisms in the final sample). Wear clean nitrile gloves while priming equipment.
 - a. Good practice is to collect both DNA and taxonomy samples while wearing clean nitrile gloves.
 - b. You may either use new gloves for each sample or re-use and clean gloves with ETOH between samples
 - i. Use a clean ETOH wipe or spritz with ETOH and wipe to remove residual material
4. Collect samples according to SOP B for the appropriate sample depth. Faster zooplankton may appear to be missing from the sample, however De Bernardi (1984) suggests that trade-offs are made with every sampler, and replicate samples collected from clear samplers improve estimates of zooplankton abundance.
5. Choose "DNA" in the mobile app, and the appropriate habitat and sampler metadata.
6. Sample ID = *SITE.YYYYMMDD.sampleType.sampleNumber.DNA*. Check that the sample ID on the label matches the sample ID in the mobile app.
7. After sample collection, use forceps or gloved hands to pick out large organic matter, leaving the zooplankton in the sample.
 - a. Samples will be homogenized in a blender at the external facility. Make sure there are no large sticks or other material in the sample that could interfere with homogenization.
8. Minimize the amount of water in the sample by using as little water as possible for rinsing.
9. Add 95% ETOH to sample jar to completely cover sample (Stein et al. 2012). Final ETOH concentration should be as close to 95% as you can get, and at least greater than 50%.
10. Return sample to domain lab and store at 4 °C.

SOP D Post-Field Sampling Tasks

1. Refresh the sampling kit
 - a. Replace sample bottles.
 - b. Print new field labels and field data sheets.
 - c. Refill/restock preservative containers.
2. Equipment maintenance, cleaning and storage
 - a. Decontaminate all equipment that has come in contact with lake/non-wadeable stream water according to the NEON Aquatic Decontamination Protocol (RD 09)].
 - b. Dry all equipment thoroughly before storage.
 - c. Check all nets for holes and patch if necessary.



3. Data QA/QC

a. Required checks

- i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles.
- ii. Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.

b. Nice to check

- i. Site ID, collect date, sampling protocol version
- ii. Sampling depths

D.1 Document Incomplete Sampling Within a Site

Zooplankton sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and Appendix C. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot is compromised after road-building activities, or a stream moves after a flood and the location is no longer within the stream channel). Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

Zooplankton sampling locations may become compromised if the water level of the lake drops too low to samples near the inlet and outlet infrastructure. If sampling at a given location is not possible during a given bout a problem ticket should be submitted by Field Science staff.

Please note that 3 zooplankton samples is the minimum that can be collected during a bout to ensure scientifically useful data. Therefore, it is preferable to collect a sample in a different location rather than not collect a sample at all.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS Sampling Incomplete: ZOO – [Root Cause Description]'
 - a. Example: 'AOS Sampling Incomplete: ZOO – Could not access inlet and outlet due to cows'
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.



SOP E Laboratory Sampling and Analysis

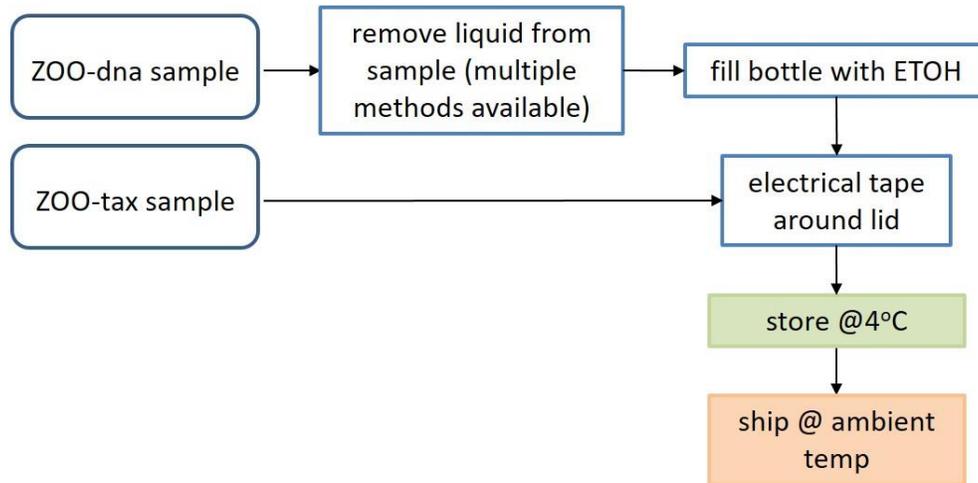


Figure 15. An expanded diagram of the lab workflow for the zooplankton lab SOP.

Preservative in DNA samples must be changed within 24 hours to preserve the integrity of DNA. This processed replaces the water in the organisms with ethanol.

1. For DNA samples (collected in SOP C), change out ethanol within 24 hours in the domain lab prior to shipping. This helps to ensure that DNA is preserved quickly and will be in good condition for analysis.
2. Decontaminate the filter apparatus, nylon mesh, and any equipment that comes in contact with the DNA sample during processing, or keep a separate, decontaminated filter apparatus for DNA.
3. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
4. Allow zooplankton to settle in the sample container, and decant or pipet ethanol off the top of the sample.
 - a. There are several ways change ETOH in zooplankton samples. Be sure that the following requirements are met regardless of which method is used:
 - i. Sample material (zooplankton) is not lost from the sample bottle. Only liquid is removed and replaced.
 - ii. Any equipment or consumables that come in contact with the sample material have been decontaminated with 10% bleach prior to the sampling day, and are rinsed with 10% bleach followed by DI between samples.
 - b. Method 1: Allow samples to settle >4 hours. Decant or pipette liquid off the top of the sample over a 63 μm sieve in case the sample spills. Return any spilled sample to the bottle, and replace liquid with ETOH.



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- c. Method 2: Use the TOS beetle filter cup with 63 μm (zooplankton) mesh (RD[13]). Rinse material on mesh into sample bottle with ETOH, but do not include in sample. The TOS beetle filter cup should screw directly onto zooplankton collection bottles.
- d. Method 3: Pour sample into dolphin bucket from the field sampling equipment. Use an ETOH-filled squirt bottle to rinse the contents of the cup back into the sample bottle. Be sure to decontaminate the dolphin bucket between samples.
5. Replace ethanol, preserving as close to 95% as possible.
 - a. Container does not need to be filled to the top with liquid as organisms will be homogenized with a blender at the external lab and do not need to remain intact.
6. Close sample container tightly.
7. ETOH wipe and sterilize filter apparatus or any equipment that comes in contact with the DNA sample material between samples.
8. Carefully clean and dry all equipment prior to storage.

E.1 Sample Storage

Store all samples at the domain support facility at 4 °C in a flammable-rated space until shipping. For shipping instructions see SOP G. If 4 °C space is an issue, DNA samples may also be stored at -20 °C until shipping.

SOP F Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription error and improve data quality. Scan barcode labels into the mobile application. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

Given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.



SOP G Sample Shipment

Information included in this SOP conveys science-based handling and packaging instructions for the samples types included in this protocol. For shipping instructions, see RD[16].

1. Keep morphological taxonomy samples and DNA samples separate.
 - a. See CLA shipping information for details on shipping locations
 - 1) Bout 2 DNA samples will be shipped to an external lab for analysis.
 - 2) Bout 1 and Bout 3 DNA samples will be shipped directly to the archive facility.
2. Tape the lids of all sample jars and bottles with electrical tape. This helps prevent leaks and ethanol fumes from fading the sample labels.
 - a. Tip: Assigning a different color of electrical tape to each site in the domain helps keep samples organized.
3. Bag samples
 - a. ZOO-tax: Place sealed sample bottles into one or several gallon-sized resealable zip-top bags, grouped by site. Sample bottles are acceptable “inner containers” required for shipping.
 - b. ZOO-dna: Individually bag each DNA sample in a zip-top bag to prevent contamination of DNA from sample to sample during shipment.
4. See RD[16] for further shipping instructions.

G.1 Handling Hazardous Material

Follow procedures for shipping ethanol in limited quantity ground shipments in AD[03] and RD[16].

G.2 Supplies/Containers

See section Appendix E and **Error! Reference source not found.** for specific shipping materials.

G.3 Timelines and Conditions

Taxonomy samples: Shipping should occur within one week of sampling if possible, however samples may be held for up to 30 days at the domain support facility if necessary. Samples may be stored at the domain support facility at 4 °C until shipping. Samples are shipped ground at ambient temperature.

DNA samples: Samples from Bout 2 must be held at 4 °C and will be shipped to the external facility for analysis on a CLA-defined schedule. Samples from Bouts 1 and 3 will be shipped directly to the archive facility on a CLA-defined schedule. Samples may be held for a maximum of 6 months if batch shipping is requested by the external lab. Samples are shipping ground at ambient temperature as this is a short enough time-period that sample degradation is not expected to occur.



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G.4 Grouping/Splitting Samples

Group samples by site per bout. Samples from multiple sites may be sent in the same shipment. Zooplankton samples may be shipped with macroinvertebrate samples if going to the same external lab.

G.5 Return of Materials or Containers

Include return shipping label with WBS code if any shipping materials need to be returned to the domain support facility (e.g., cooler). Sample bottles will not be returned.

G.6 Shipping and Chain of Custody

The mobile shipping applications are used to create chain of custody documents. Shipments are to include a hardcopy of the shipping manifest (RD[11]) in each box as well as an electronic shipping manifest that is emailed to the receiving laboratory and NEON Collections and Laboratory Analysis at the time of shipment. The shipping manifest must accurately document the physical samples inside the shipping container.

G.7 Laboratory Contact Information and Shipping/Receipt Days

See the Shipping Information for External Facilities on NEON's CLA intranet site.



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APPENDIX A QUICK REFERENCES

Step 1 – Check the zooplankton field sampling kit to make sure all supplies are packed.

Step 2 – Prepare labels (2" x 4") and barcode labels. Sample ID =
SITE.DATE.samplerType.sampleNumber[.DNA]

Step 3 – Ensure the General (AOS) Field Metadata and Gauge Height (RD[06]) and (AOS) Secchi (RD[12]) apps are completed per field site visit.

Step 5 – Determine which sampler to use based on water depth.

1. If water depth is ≤ 4 m use a Schindler-Patalas sampler.
 - a. If 2-4 m collect samples at 0.5 m, mid-water column, and 0.5 m above the lake bottom and integrate.
 - b. If 1-2 m collect sample from 0.5 m and 0.5 m above the lake bottom and integrate.
 - c. If water is < 1 m collect 2 Schindler traps 0.5 m below water surface and integrate.
2. If water depth is > 4 m, use the tow net and integrate at least 2 tows.

Step 6 – Collect 1 integrated sample for taxonomy and 1 integrated sample for DNA at:

1. In the deepest point in the lake (buoy), determined by site map and pre-loaded GPS coordinates
 - a. Location ID = "c0" or "c1" (depends on stratification)
2. Near the major inlet in the littoral zone
 - a. Location ID = "in"
3. Near the outlet in the littoral zone
 - a. Location ID = "ot"

Step 7 – Preserve samples in the field with ethanol (taxonomy final concentration 30-40%, DNA final concentration $\sim 95\%$). Replace ETOH in DNA samples within 24 hours of collection.

Step 8 – Ship samples to zooplankton taxonomy lab, zooplankton DNA lab, or archive facility.



APPENDIX B REMINDERS

Before heading into the field:

- Collect and prepare all equipment including labels and barcodes.
- Check for holes in nets, assure that nets are clean and free of debris and organic matter and have been decontaminated (RD[08]) since last use.
- Sterilize all equipment for DNA collection.

Sample collection:

- When making contingent decisions, a suite of 3 samples is the minimum number of samples in order to do statistics on the data. Do not collect fewer than 3 samples.
- Choose the appropriate sampler based on depth.
- Always sample near the bow of the boat to minimize the effects of the motor on the water column.
- Use deionized water, tap water, or 0.63 μm filtered lake water to rinse inside zooplankton sampling nets to prevent introduction of additional zooplankton to the samples. Do not introduce additional zooplankton to sample from rinsewater.
- Take care not to submerge the top of the net opening below the water surface after collecting the sample, during rinsing.
- Zooplankton often become trapped in the folds of the nets (near the stitching). Check net seams between samples to ensure that specimens are added to the correct samples, and do not remain in the net between sites.
- Wear gloves and use methods that are as sterile as possible for DNA collection.

Sample processing:

- Preserve samples in the field within 30 minutes of collection.
- Store at 4 °C until shipping to the external lab.
- Change ETOH in DNA samples after collection.

Data QA/QC:

Required checks

- Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles.
- Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.

Nice to check

- Site ID, collect date, sampling protocol version
- Sample depths



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APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes. General guidelines for developing these dates are presented in the NEON Aquatic Sample Strategy Document (RD[08]). Also see the Site Specific Sampling Strategy Documents for each domain (RD[15]).

Domain	Site		Bout 1	Bout 2	Bout 3
D03	Lake Barco	BARC	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	SUGG	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D05	Crampton Lake	CRAM	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D05	Little Rock Lake	LIRO	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D09	Prairie Lake	PRLA	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	PRPO	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D18	Toolik Lake	TOOK	21May-18Jun	29Jun-27Jul	6Aug-3Sep



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APPENDIX D SITE-SPECIFIC INFORMATION: SAMPLER RECOMMENDATIONS

Sampler types should be consistent at a location from year to year.

Domain	Site		Buoy (c0)	Inlet (in)	Outlet (ot)
D03	Lake Barco	BARC	tow net	Schindler	Schindler
D03	Lake Suggs	SUGG	Schindler	Schindler	Schindler
D05	Crampton Lake	CRAM	tow net	Schindler	Schindler
D05	Little Rock Lake	LIRO	tow net	Schindler	Schindler
D09	Prairie Lake	PRLA	Schindler*	Schindler	Schindler
D09	Prairie Pothole	PRPO	Schindler*	Schindler	Schindler
D18	Toolik Lake	TOOK	tow net	Schindler	Schindler

*May use tow net when water is high.



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APPENDIX E EQUIPMENT

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 6. Equipment list – General equipment

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
RD[10]	N	Site-specific Bathymetry Map	Determining sampling locations	1
	N	Work gloves	Used with the samplers for safe handling of the rope	1 pair
CDW-G: 4452963	N	Mobile data entry tablet	Field data entry	1
	N	Cooler with ice packs	Keeping samples cool	1
Consumable items				
Forestry Suppliers, Inc.: 010510-1 49247	N	Aquatic Field Metadata Sheet and Field data sheets (all-weather paper)	Recording metadata if tablet fails	1
Forestry Suppliers, Inc.: 010510-1 49247	N	Pre-printed adhesive labels	Labeling sample bottles, human-readable	1 sheet
	N	Adhesive barcode labels (Type I)	Labeling sample bottles with barcode-readable	1 sheet
	N	Laboratory nitrile gloves	Preventing preservative contact with skin	3 pair
	N	Pencils	Recording data	4
Grainger, W.W.: 1JU51	N	Permanent markers	Labeling samples	4



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Table 7. Equipment list – Sampling equipment

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
Forestry Suppliers, Inc.: 426A10	N	Plankton tow net, 12" mouth, 63 µm mesh	Collecting samples >4m water depth	1
	N	Nylon safety line (10-50 m)	Used with tow net and Schindler-Patalas	1
Forestry Suppliers, Inc.: 217004	R	Schindler-Patalas sampler, 12 L, 63 µm mesh size	Collecting samples ≤4 m water depth	1
Fisher Scientific Company: 0340910E	N	500 mL Wash bottle	Rinsing the sampler net	1
BioQuip Products Inc.: 1426B Fisher Scientific Company: NC0095946	N	Plastic sampling tray	Removing debris from the sample	1
BioQuip Products Inc.: 4748	N	Forceps	Removing debris from the sample	1
Amazon Capital Services Inc.: B00X0WT8MQ	N	Depth finder	Determining depth at sampling location	1
Consumable items				
Fisher Scientific Company: 033134E	Y	500 mL Nalgene sample bottles	Sample container	3
Thomas Scientific, Inc.: 1709C04	Y	1 L Nalgene sample bottles	Sample container for large samples	3
Fisher Scientific Company: 4355601 Thomas Scientific, Inc.: C954K61	N	Ethanol, 95%, undenatured	Preservative	1
	N	DI water or tap water	Rinsing	2L
Grainger, W.W.: 5LH30	N	Resealable bags (gallon)	Organizing sample bottles	3



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Table 8. Equipment list – Laboratory processing (DNA samples)

Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
Forestry Suppliers, Inc.	N	Sieve, 63 um	Changing preservative	N
Fisher Scientific Company: 0340910E	N	500 mL Wash bottle, for ETOH	Rinsing samples with ETOH	1
Consumable items				
Amazon Capital Services Inc.	N	63 um Nitex mesh cloth, cut into squares for filter cup	Catching zooplankton over the filter cup	N
Fisher Scientific Company: 4355601 Thomas Scientific, Inc.: C954K61	N	Ethanol, 95%, undenatured	Preservative	1
Fisher Scientific Company, LLC: 033134E Amazon Capital Services Inc.: 24-C44	N	Filter cup (500 mL HDPE bottle and 63 um Nitex mesh square, see TOS beetle protocol)	Changing preservative	1
	N	Chlorine bleach (6%)	Decontaminating DNA equipment	1 gal
	N	Nitrile gloves	Maintaining sterile conditions	N